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Peptide-rich extracts from spent yeast waste streams as a source of bioactive compounds for the nutraceutical market

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ABSTRACT

Spent yeast is one of the main fermentation process by-products and several bioactive ingredients have been extracted from it, such as mannans and β-glucans. These extraction processes generate waste streams rich in protein, that can still be used to produce peptide-rich extracts. This work describes the use of a scalable and low-cost process to obtain yeast peptide extracts in a circular economy concept. Peptide fractions produced contained 48.3 to 86.4% of protein (*w*/w) and essential amino acids amounts higher than those defined by FAO and WHO references. Regarding their bioactive properties, extracts exhibited strong antihypertensive and antioxidant activities. Furthermore, an inhibition of 62.0–71.3% in HMG-CoA reductase was observed, a feature hereby described for the first time, thus stating the cholesterol-lowering capacity of yeast peptides. In conclusion, due to their combined nutritional and bioactive characteristics, the produced yeast peptide extracts are good candidates for dietary supplementation and functional foods.

1. Introduction

The growth of brewing industry generates increasing amounts of spent yeast from its fermentation processes, being one of its main by-products (Puligundla, Mok, & Park, 2020). Using the beer production data from 2020 (around 1.82 billion hectoliters), and considering the production of 1.7 to 2.3 g spent yeast per litre, spent yeast production ranged between 309,400 to 418,600 t on 2020 (Conway, 2021; Marson, de Castro, Belleville, & Hubinger, 2020). Spent brewer's yeast is described as a Generally Recognized As Safe (GRAS) microorganism (Rakowska, Sadowska, Dybkowska, & Świderski, 2017) and has been sold as yeast extract for more than a century (Ritala, Häkkinen, Toivari, & Wiebe, 2017).

Spent yeast is mainly constituted by proteins, including essential amino acids with high biological value, representing about 45 to 60% of its dry weight (Vieira, Cunha, & Ferreira, 2019). Capsular and cell wall glucans, mannans and chitin constitute the polysaccharide fraction, which represent about 25–35% of the yeast, followed by 5–10% of glycoproteins (mannoproteins from cell wall and functional enzymes). Nucleic acids (4–8%), lipids (4–7%) and polyphosphates (1–3%) are the components present in lower amounts (Feldmann, 2012).

Several methods for mannans and β-glucan extraction from yeast have been described (Freimund, Sauter, Käppeli, & Dutler, 2003; X. Y. Liu, Wang, Cui, & Liu, 2008; Tian, Yang, & Jiang, 2019), since these molecules present numerous promising bioactivities such as prebiotic and bacterial pathogen inhibition for mannans (Faustino, Durão, Pereira, Pintado, & Carvalho, 2021), and mainly immunopharmacological properties for β-glucan (Avramia & Amariei, 2021). Generally, these extraction processes start with yeast cell lysis, followed by separation of the released target compounds from the remaining cell components. Proteins are the main remaining cell component and for this reason, waste streams of these extraction processes are usually rich in soluble protein, that can be further processed to recover proteins and peptides in a circular economy-based approach.

Membrane filtration technology has been widely used for separation and concentration of spent yeast protein and bioactive peptides, since it can be operated under gentle conditions, allowing cell debris removal with low energy consumption and without compromising molecular

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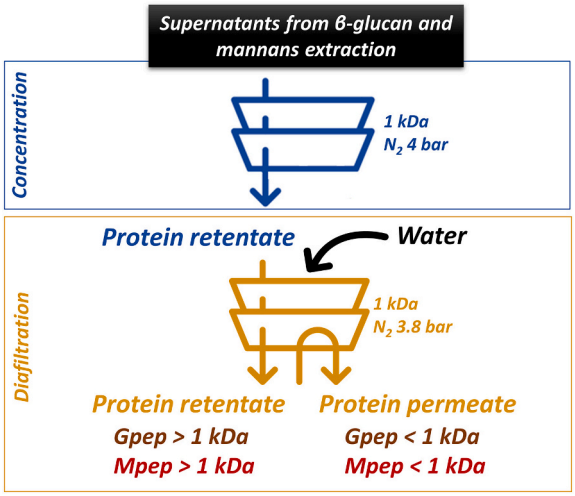


Fig. 1. Diagram for ultrafiltration process of waste streams of β-glucan and mannan extraction processes from spent yeast to obtain peptide-rich extracts.

structures, and consequently, their biological activities (Vollet Marson, Belleville, Lacour, & Dupas Hubinger, 2020). Depending on protein and peptides molecular weight (MW) and the intended application, different membrane filtration cut-offs and materials can be used in order to obtain the desired product (D. Liu, Savoire, Vorobiev, & Lanoisellé, 2010). Frequently, this technique is applied after yeast protein extraction or prior to other protein purification methods, such as chromatography (Bartlomiej Podpora, Swiderski, Sadowska, Rakowska, & Wasiak-Zys, 2016). Furthermore, this technology has been widely used in food industry for processing new ingredients and foods due to its green characteristics (Dhineshkumar & Ramasamy, 2017).

Meanwhile, cosmeceutical and nutraceutical sectors have also been working on yeast bioactive peptides mainly because of their antihypertensive (Amorim et al., 2019) and antioxidant (Guo, Guo, & Liu, 2020) features that have been related with development of cardiovascular diseases (Mirzaei, Mirdamadi, Ehsani, Aminlari, & Hosseini, 2015). Antidiabetic potential of yeast extracts has also been reported (Hu, Wang, Guo, Li, & Hou, 2014) as well as anti-stress effects (Lee, Jung, & Suh, 2009), *in vitro* immunostimulation (Williams, Dias, Jayasinghe, Roessner, & Bennett, 2016) and *in vivo* influence in bone growth (Lee et al., 2011).

Due to the increasing interest in yeast peptides, the aim of the present study was: (i) to assess the feasibility of obtaining peptide-rich extracts from spent yeast waste streams of β-glucans and mannans extraction processes, using membrane filtration, and (ii) to evaluate the potential of those peptide-rich extracts to be used as ingredients for the nutraceutical market, thus measuring their antihypertensive, antioxidant and anti-cholesterolemic potential. To our knowledge, the present study assessed the effect of peptides from spent yeast in targeting cholesterol levels through HMG-CoA reductase inhibition for the first time.

2. Material and methods

2.1. Peptide-rich extracts production by selective membrane ultrafiltration

Peptide-rich extracts were obtained from waste streams of β-glucan (Gpep) and mannan (Mpep) extraction (Freimund et al., 2003; X. Y. Liu et al., 2008; Tian et al., 2019) from engineered spent yeast (*S. cerevisiae*) used in Amyris facilities to produce farnesene (Emeryville, California, USA). Liquid stream (supernatants) underwent 1 kDa cut-off membrane ultrafiltration in an Amicon® stirred cell model (Merck KGaA, Darmstadt, Germany) in order to get peptide rich fractions with different MW: Gpep >1 kDa, Gpep <1 kDa, Mpep >1 kDa and Mpep <1 kDa. As described in Fig. 1, supernatant was submitted to ultrafiltration with a Ultracel® 1 kDa ultrafiltration discs (regenerated cellulose, 76 mm diameter) (Merck KGaA, Darmstadt, Germany) in order to concentrate the protein in the retentate. Thereafter, the retentate was diafiltrated with 3 volumes of deionized water to further purify the obtained fractions. At the end of ultrafiltration process, samples were freeze-dried (Freeze-dryer Alpha 2–4 LSCbasic,Martin Christ, Osterode am Harz, Germany).

2.2. Chemical characterization of peptide fractions

2.2.1. Nutritional analysis

2.2.1.1. Protein and dry weight quantification. Protein content of fractions was determined by Pierce™ BCA protein assay kit (Thermo Fisher Scientific Inc., Massachusetts, USA). The method is based on reduction of Cu^{2+} to Cu^{1+} by protein in alkaline medium, followed by Cu^{1+} reaction with bicinchoninic acid (BCA), which produces a purple-coloured product that can be read at 562 nm after 30 min of reaction; assays were performed in 96 well microplates in a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA).

Dry weight was determined at 105 °C for 24 h according to standard procedures of the Association of Official Analytical Chemists (AOAC, 2005).

2.2.1.2. Free and total amino acids quantification. The amino acids profile was analysed by iodoacetic acid derivatization and o-phthaldialdehyde methodology, using reverse phase high performance liquid chromatography with a Chromolith® Performance RP18 (4.6 × 100 mm) column (Merck KGaA, Darmstadt, Germany) for separation, and coupled to a high resolution fluorescence detector (Agilent Technologies, Inc., California, USA).

For determination of total amino acids, an acid hydrolysis during 20 h at 115 °C was performed at 10 mg of peptide fraction in 3 mL HCl 6 M before HPLC analysis. Nitrogen was injected through an inlet needle to substitute samples atmosphere and another needle was used as gas outlet. Flasks were sealed with tape and placed in an oven at 115 °C for 20 h. Then, pH was adjusted to 3.2 and the solution diluted rigorously to a final volume of 10 mL (Wang et al., 2016). For quantification of free amino acids, peptide fractions were prepared in HCl 0.1 M at concentration of 10 mg/mL. According to procedure of Pripis-Nicolau, De Revel, Marchand, Beloqui, and Bertrand (2001), 20 μL of peptide fraction was derivatized and 10 μL was injected. The analysis was done in triplicate and the amino acids quantified according to calibration curves of pure standards (aspartic acid (Asp), glutamic acid (Glu), cysteine (Cys), asparagine (Asn), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys)) (Sigma-Aldrich, Inc., St. Louis, USA) from 1 to 30 mg/L, using norvaline (Sigma-Aldrich, Inc., St. Louis, USA) as internal standard.

2.2.1.3. Minerals. Mineral content of peptide-rich extracts was measured in an optical emission spectrometer Model Optima 7000 DV ™ ICP-OES (Dual View, PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with radial configuration according to procedure of Chatelain, Pintado, and Vasconcelos (2014). The analysis was done in triplicate and the minerals quantified according to calibration curves of a commercial mix standards for ICP analysis (Inorganic Ventures, Christiansburg, USA) (molybdenum, zinc, cadmium, phosphorus, lead, nickel, cobalt, boron, manganese, iron, magnesium, calcium, copper, aluminium, sodium and potassium) from 0.05 to 10 mg/L. Before ICP analysis, a microwave digestion of samples (2 mL) was performed in a speedwave XPERT (Berghof Products + Instruments GmbH, Eningen, Germany) using 5 mL of Suprapur® HNO_3 and 2 mL of 35% H_2O_2 (Merck KGaA, Darmstadt, Germany).

2.2.2. Molecular weight distribution

A preliminary MW peptide evaluation of original Gpep and Mpep supernatants (non-treated waste streams) was performed by fast protein liquid chromatography (FPLC) to select the cut-off of ultrafiltration membrane. A two-column set composed by a Superdex® 200 Increase 10/300 GL and a Superdex® peptide 10/300 GL columns (Cytiva, USA) coupled to an AKTA Pure 25 purification (Cytiva, USA) was exploited and a 0.05 M phosphate buffer pH 7.0, containing 0.15 M sodium chloride and 0.2 g/L of sodium azide, was used as mobile phase at a flow rate of 0.5 mL/min. The elution was monitored at 280 nm and thyroglobulin (669 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovoalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa), whey peptide (1.2 kDa) and tryptophan (180 Da) were used as standards for calibration curve.

The evaluation of MW peptides distribution at Gpep and Mpep fractions was performed on an ultra-high-performance liquid chromatography system from Bruker Elute series, coupled to an ultrahigh-resolution quadrupole−quadrupole time-of-flight (UHR − QqTOF) mass spectrometer (Impact II; Bruke Daltonik GmbH, Bremen, Germany) using an Intensity Solo 2 C18 (100 × 2.1 mm, 2.2 μm, Bruker Daltonik GmbH, Bremen, Germany) (BRHSC18022100) set for 60 °C. The mobile phases used were 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B) at 0.250 mL/min flow rate in gradient mode: 95% A and 5% B until 14 min, 5% A and 95% B from 14 to 22 min, and 95% A and 5% B until reaching the run end (25 min). Instrument was operated in MS positive mode and data was collected in the range of 150 to 2200 *m/z*. The selected parameters were as follows: capillary voltage, 4.5 kV; drying gas temperature, 220 °C; drying gas flow, 9.0 L/min; nebulizing gas pressure, 0.6 bar; collision radio frequency, 2000 Vpp; transfer time, 90 μs; and pre-pulse storage, 10 μs. Post-acquisition internal mass calibration used ESI-L Low Concentration Tuning Mix (Agilent Technologies Inc., CA, USA) delivered by a syringe pump at the start of each chromatographic analysis.

2.3. Biological activity

2.3.1. In vitro cytotoxicity assessment

2.3.1.1. Cell culture

2.3.1.1.1. Caco-2. Human colon carcinoma (Caco-2) cells were obtained from the European Collection of Authenticated Cell Cultures and were grown at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 using high glucose (4.5 g/L) Dulbecco's Modified Eagle's Medium supplemented with 10% (*v*/v) heat inactivated fetal bovine serum (Thermo Fisher Scientific, MA, USA), 1% (v/v) penicillin–streptomycin–fungizone (Lonza, Verviers, Belgium), and 1% (v/v) of non-essential amino acids 100× (Sigma-Aldrich, St. Louis, USA). Cells were used between passages 26 and 29.

2.3.1.2. Cytotoxicity assay. Cytotoxicity evaluation was performed according to the ISO 10993-5:2009 standard (ISO, 2009). The cells were seeded at 1×10^5 cells/well in a 96-well microplate and after overnight incubation at 37 °C, they were treated with different concentrations of Gpep and Mpep fractions (from 0.8 to 5 mg/mL) prepared in fresh culture medium. DMSO (Sigma-Aldrich, St. Louis, USA) at 10% (v/v) in culture media was used as a cell-death control and unseeded culture media was used as a growth control. After 24 h-exposure, the PrestoBlue® reagent (Invitrogen, Massachusetts, USA) was added to each well and incubated for 2 h. The fluorescence was recorded (excitation 570 nm; emission 610 nm) after incubation using a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA). All assays were performed in quadruplicate.

2.3.2. Angiotensin converting enzyme (ACE) inhibition assay

The ACE-inhibitory activity was evaluated according to Amorim, Marques, et al. (2019). The reaction used *o*-Abs-Gly-*p*-nitro-Phe-OH trifluoroacetate salt (Bachem, Bubendorf, Switzerland) as substrate and 42 mU/mL of ACE (peptidyl-dipeptidase A from rabbit lung) (Sigma-Aldrich, St Louis, USA) at pH 8.3 with 0.1 mM zinc chloride (Sigma-Aldrich, St Louis, USA). A black polystyrene 96-well microplate (Thermo Fisher Scientific, MA, USA) was used and the incubation was carried out at 37 °C in a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA), for 30 min, recording the fluorescence in kinetic mode, using 350 nm for excitation and 420 nm for emission. The percentage of ACE inhibition was calculated by the following formula:

$$\text{ACE inhibition } (\%) = \frac{\left(F_{control} - F_{sample}\right)}{F_{control}} \times 100$$

where $F_{control}$ and F_{sample} are the fluorescences of control (maximum ACE activity) and sample, respectively. The calculation of IC50 values (concentration needed to inhibit 50% of ACE activity) for all four fractions was performed by non-linear fitting of the data, using a four-parameter logistic regression model. The assay was performed in duplicate.

2.3.3. Antioxidant capacity

2.3.3.1. Scavenging activity using 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulphonate)-radical ($ABTS^+$). For measuring the antioxidant activity of peptides, 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulphonate)-radical ($ABTS^+$) scavenging activity was performed according to Gonçalves et al. (2019) with some modifications for a 96-well microplate scale. The cation-radical $ABTS^+$ was generated by reaction of the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) and potassium persulphate after incubation at room temperature in the dark for 16 h. Then, $ABTS^+$ stock solution was filtered with a 0.45 μm syringe filter and daily diluted to prepare a working solution with an absorbance of 0.70 ± 0.02 at 734 nm. A standard curve of Trolox (50–560 μM) was also prepared daily. A black polystyrene 96-well microplate (Thermo Fisher Scientific, MA, USA) was used for the reaction where 15 μL of sample, Trolox or solvent was added to 200 μL $ABTS^+$ working solution. The incubation was carried out at 30 °C in a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA) during 5 min and the absorbance at 734 nm was read upon completion. The $ABTS^+$ scavenging activity percentage was calculated by the following formula:

$$ABTS^+ \text{ scavenging activity } (\%) = \frac{\left(A_{Control} - A_{sample}\right)}{A_{control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of control and sample, respectively. The assay was performed in triplicate. The Trolox standard curve was used to express Trolox Equivalent (TE) antioxidant activity of extracts (μmol/ g extract).

2.3.3.2. Scavenging activity using 2,2-diphenyl-1-picrylhydrazyl-free-radical (DPPH). Additionally, 2,2-Diphenyl-1-picrylhydrazyl (DPPH)-free-radical scavenging activity was assessed according to Prior, Wu, and Schaich (2005) with some modifications for a 96-well microplate scale. A 600 μM stock solution of DPPH was prepared in methanol (stored in the dark at −20 °C) and diluted prior to use to 60 μM by adjusting the absorbance to 0.600 ± 0.100 at 515 nm (working solution). A standard curve of Trolox (7.5–240 μM) was also daily prepared. A black polystyrene 96-well microplate (Thermo Fisher Scientific, MA, USA) was used from mixture reaction where 25 μL of sample, Trolox or solvent was added to 175 μL DPPH working solution. The incubation was carried out at 25 °C in a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA) during 30 min and the absorbance at 515 nm was read in the end. The DPPH scavenging activity percentage was calculated by the following formula:

Table 1
Protein concentration (% *w*/w), sugars (% w/w) and minerals (ng/g extract) of peptide-rich extracts.

	Protein (%)	*Sugars (%)*	*Minerals (ng/g)*					
			P	*Mg*	*Ca*	*Na*	*K*	*Total*
Gpep > 1 kDa	67.2 ± 16.0	28.2 ± 7.9	4.20 ± 0.03	1.52 ± 0.01	2.31 ± 0.02	ND	3.32 ± 0.02	11.4 ± 0.1
Gpep < 1 kDa	67.6 ± 25.6	4.34 ± 1.73	13.8 ± 1.3	8.20 ± 0.50	7.91 ± 0.34	0.673 ± 0.101	37.0 ± 3.2	67.5 ± 5.5
Mpep > 1 kDa	86.4 ± 8.7	7.44 ± 0.22	5.22 ± 0.01	1.04 ± 0.01	0.100 ± 0.010	7.63 ± 0.01	13.0 ± 0.01	27.0 ± 0.01
Mpep < 1 kDa	48.3 ± 15.9	3.32 ± 1.06	9.39 ± 0.01	2.07 ± 0.01	0.189 ± 0.010	41.8 ± 0.01	64.7 ± 0.01	118 ± 0.01

Results are expressed in average ± standard deviation ($n = 2$). ND – Not detected (below low detection limit), P – Phosphorus, Mg – Magnesium, Ca – Calcium, Na – Sodium, K –Potassium. ´

$$\text{DPPH scavenging activity } (\%) = \frac{\left(A_{Control} - A_{sample}\right)}{A_{control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of control and sample, respectively. The assay was performed in triplicate. The Trolox standard curve was used to express TE antioxidant activity of extracts (μmol/ g extract).

2.3.4. HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibition assay

The HMG-CoA reductase inhibition assay was determined by HMG-CoA Reductase Activity Assay Kit (colorimetric) (Abcam, Cambridge, United Kingdom). The assay was performed according to the manufacturer's instructions. This method is based on the consumption of NADPH by the enzyme which can be measured by the decrease of absorbance at 340 nm. The limit of detection is below 0.05 mU of enzyme. The reaction was monitored for 15 min in a microplate reader (Synergy H1, Biotek Instruments, Winooski, USA) and the absorbance values plotted against time. As recommended in the kit, the absorbance of two time points within the linear range and with a minimum of 2 min apart were selected and used in the calculations of the HMG-CoA reductase activity (units/mg protein) and the HMG-CoA reductase inhibition (%). A reference drug inhibitor, Atorvastatin (provide in kit) and a commercial inhibitor, Pravastatin, were used as control. The samples and controls were assessed in triplicates in 2 different plates using 2 sets of the same samples, prepared fresh but in different occasions.

2.3.5. Statistical analysis

Results are expressed as mean ± standard deviation from assay replicates. Statistical analysis was performed using the GraphPad Prism 6 software (GraphPad Software, CA, USA). Normality of data was tested using the Shapiro-Wilk's test and the comparison between different peptide-rich fractions were performed using the one-way ANOVA followed by Tukey's multiple comparisons test.

3. Results and discussion

3.1. Nutritional analysis

The supernatants of extraction of β-glucan (Gpep) and mannan (Mpep) processes were collected in order to produce peptide-rich extracts in a circular economy-based approach. Membrane filtration technology has been described as an important tool for separation of soluble intracellular proteins from cell lysates based on sieving and charge-based mechanisms (Vollet Marson et al., 2020). High selectively and efficiency combined with low energy consumption are the main reasons for its use in food processing industry, being specially chosen for peptides and proteins because of gentle treatment characteristics (Mohammad, Ng, Lim, & Ng, 2012). In fact, it is very important to preserve peptide structure and physicochemical attributes, since their bioactivities are described as to be highly dependent on them (Marson, de Castro, Belleville, & Hubinger, 2020). According to size of proteins and their fractions of interest, ultrafiltration is the main pressure-driven process used because of its range of membrane MW cut-offs (Vollet Marson et al., 2020).

To select the ultrafiltration membrane MW cut-off, a preliminary MW evaluation was performed by fast protein liquid chromatography (FPLC), where it could be observed that the majority of peptides obtained approximated 1000 Da (*Supplementary material 1*). After protein concentration at 1 kDa, a diafiltration was executed in order to purify the final extracts. At the end of membrane filtration, they were dried by freeze-drying. This process resulted in four protein rich extracts with different MW and nutritional characteristics: Gpep >1 kDa, Gpep <1 kDa, Mpep >1 kDa and Mpep <1 kDa (Table 1). The protein concentration in Gpep and Mpep fractions increased to 48 to 86%, respectively (Table 1), with a protein yield from 0.4 to 2.2 g per litter of supernatant processed. Gpep waste stream allowed the higher amount of protein yield (Gpep >1 kDa: 2.2 ± 0.5; Gpep <1 kDa: 1.8 ± 0.4 g/L supernatant) in comparison with Mpep (both 0.4 ± 0.1 g/L supernatant). Concerning about protein concentration, Mpep >1 kDa had the highest value of the four fractions since both extracts from Gpep rounded the same protein amount (Table 1).

A small sugar content was determined in all peptide extracts (3–7%) (except Gpep >1 kDa) (Table 1), as expected, since we are working on waste streams from polysaccharides extraction. The extract with the higher sugar content (Gpep >1 kDa) can contain some oligosaccharides, since β-glucan and mannans extraction processes are distinct, and probably generate different sizes of saccharides.

Several authors have used membrane filtration technique to produce peptide fractions (Amorim et al., 2016; Amorim, Pinheiro, & Pintado, 2019; Hu et al., 2014; Marson et al., 2021; Marson, Lacour, Hubinger, & Belleville, 2022; Mirzaei, Mirdamadi, & Safavi, 2019) from spent yeast, such as *S. cerevisiae* or *S. pasturianus*. By applying a ultrafiltration process with a 10 kDa and 3 kDa MW cut-off on spent yeast hydrolysates, Amorim et al. (2016) obtained a range of four fractions with protein concentration from 30 to 70%. In comparison, the present study achieved a higher protein purity. On the other hand, the authors obtained higher sugar concentrations (27 to 48%), with the highest amounts in fractions above 3 kDa, which was not observed in the current work. However, the differences observed may be related with spent yeast source, since the raw material of the current investigation is not the original spent yeast (as used by Amorim et al. (2016)), but waste streams from polysaccharides (β-glucan and mannans) extraction processes. Since these molecules are the target polysaccharides within their corresponding extraction processes, their waste streams will be poor in sugars and rich in proteins. A 3 kDa ultrafiltration was also used by Amorim, Pinheiro, and Pintado (2019) after yeast autolysis and hydrolysis for production of peptide fractions with ACE inhibitory activity, but the protein content of extracts did not exceed 40%. Similar protein and sugars contents to those of Gpep fractions were obtained by Jung et al. (2011) on a high-Cyclo-His-Pro content yeast hydrolysate (64.9% of protein and 26.9% of carbohydrates) while using a 10 kDa ultrafiltration process. In fact, the yeast hydrolysate revealed antioxidant and antidiabetic properties, since the authors found strong scavenging activity together with significant decrease in glucose level of mice hyperglycemic models. For this reason, and the potential ACE inhibitory effects shown by Amorim, Pinheiro, and Pintado (2019), the peptide-rich fractions of the current investigation were also evaluated in terms of

Table 2
Essential amino acids content (mg/g protein) of peptide-rich extracts.

	Gpep > 1 kDa	*Gpep < 1 kDa*	*Mpep > 1 kDa*	*Mpep < 1 kDa*	*FAO/WHO reference* [b]
His	22.8 ± 12.2	19.5 ± 12.8	25.8 ± 11.9	10.6 ± 8.0	15.0
Thr	35.2 ± 9.3	27.6 ± 5.2	49.2 ± 11.1	15.5 ± 8.8	11.0
Arg	28.1 ± 6.0	18.9 ± 2.6	35.4 ± 8.6	14.2 ± 7.5	NM
Val	34.2 ± 10.9	44.5 ± 11.8	64.2 ± 24.2	21.6 ± 1.8	15.0
Phe	14.5 ± 5.3	20.8 ± 6.2	32.1 ± 12.4	9.37 ± 0.53	21.0[c]
Tyr[a]	15.5 ± 3.8	15.8 ± 5.9	29.0 ± 6.7	24.8 ± 23.0	
Ile	24.5 ± 8.4	30.8 ± 6.2	44.2 ± 15.1	12.6 ± 1.8	15.0
Leu	29.4 ± 8.3	41.0 ± 6.8	57.8 ± 13.0	17.0 ± 3.0	21.0
Lys	46.3 ± 9.8	35.6 ± 8.5	59.0 ± 16.4	17.7 ± 5.6	18.0
Total EAA	*235 ± 70*	*239 ± 60*	*368 ± 113*	*119 ± 37*	*136*

Results are expressed in average ± standard deviation (n = 2).
EAA – Essential amino acids, NM – Not mentioned, His – Histidine, Thr – Threonine, Arg – Arginine, Ala – Alanine, Val – Valine, Phe – Phenylalanine, Ile – Isoleucine, Leu - Leucine, Lys – Lysine, Tyr - Tyrosine.
[a] Non-essential.
[b] World Health Organization (World Health Organization, 2007).
[c] Phe + Tyr.

potential biological activity to be applied in the nutraceutical market.

Other authors have applied multi-step sequential membrane filtration technology with different cut-off membranes to spent yeast hydrolysates. At 15–8-1 kDa process, Marson et al. (2022) obtained a 8 kDa retentate with 68.6% of protein purity, as observed in our Gpep fractions, although the respective sugar amount was lower (5.7%). On the other hand, the 1 kDa retentate and permeate showed only 25.8% and 18.5% of protein content, respectively, which are lower values from the present study. At 50–8-1 kDa ultrafiltration, the 1 kDa fractions presented protein values from 31.7 to 46.2% which are near Mpep <1 kDa result. These differences can be mainly related with the retention of polysaccharides and proteins at the first filtration processes (50, 15 and 8 kDa) which were not applied in the present study. In fact, a sequential filtration process seems to be a good choice for protein purification in spent yeast hydrolysates since in all ultrafiltration steps undesirable compounds for food industry were retained (sugars and RNA) but the protein yield of the process can be compromised. In comparison, and given our more cleaner starting matrix, the use of a single filtration step seems more efficient for obtaining high purity protein extracts.

Concerning mineral concentrations, fractions <1 kDa presented the higher concentrations (67–118 ng/g) (Table 1), in particular Mpep <1 kDa with an excess of sodium (Na) and potassium (K), which can be related with the addition of saline solutions in some steps of the extraction process (Freimund et al., 2003). A similar result was found by Amorim et al. (2016), since the permeate fractions presented a higher total amount of minerals than the retentates, namely in Na and K. These authors suggest their use for an alternative to common salt or as mineral enhancer in food, given that the higher content in K compared to Na can prevent cardiovascular diseases and high blood pressure. Regarding the recommended salt amount per day (< 5 g, corresponding about 2 g of Na), Na concentration of protein-rich extracts of the present study did not exceed the World Health Organization (WHO) limit based on controlled doses consumption (World Health Organization, 2012).

Spent brewer's yeast has been described as a potential source of essential amino acids (EAA) (Jacob, Hutzler, & Methner, 2019; Puligundla et al., 2020), as spent yeast amino acid content reported values are within Food Agriculture Organization of the United States (FAO) and WHO recommendations (Marson, de Castro, Belleville, & Hubinger, 2020). In our extracts, the amino acid profiles were traced by quantification of total and free amino acids (*Supplementary material 2*). Despite tryptophan (Trp) being an EAA, the applied analytical method was not suitable for its quantification. The results of the other EAA determined were presented in Table 2, and a range from 119 to 235 mg/g protein of extract can be observed. The total EAA of Gpep >1 kDa, Gpep <1 kDa and Mpep >1 kDa exceeded the FAO/WHO recommendation (World Health Organization, 2007), as well as individual EAA in all fractions (except histidine, leucine and lysine in Mpep <1 kDa) which makes the obtained peptide-rich extracts of present study good candidates for dietary supplementation and functional foods.

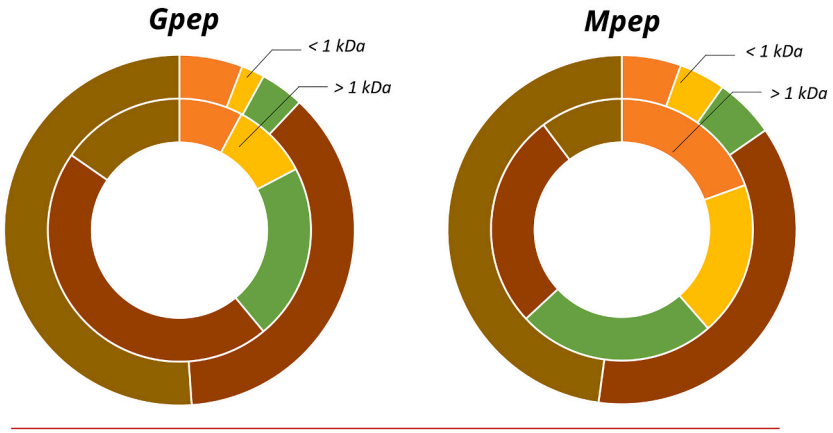
Regarding the non-essential amino acids (*Supplementary material 2*), the high amount of glutamic acid (58.4 to 111 mg/g protein) increases the potential application of these extracts in the food market since glutamic acid leads to strong flavour-enhancing properties. In fact, monosodium glutamate, a salt form of glutamic acid, is one of the well-known flavour ingredients used by food industry since it provides the typical "umani" flavour. Recently, its use has become a strategy for common salt substituent in foods as well (Maluly, Arisseto-Bragotto, & Reyes, 2017). For athletic performance, alanine supplementation has become a common practice among competitive athletes since its mechanism is involved in delaying the fatigue during high-intensity exercise (Hoffman, Varanoske, & Stout, 2018).

The quantification of free amino acids showed that the fractions <1 kDa had a higher amount (121 to 243 mg/g protein) than >1 kDa (8 to 13 mg/g protein), since amino acids in their free form have low MW and easily concentrate in ultrafiltration permeate (*Supplementary material 2*). Amorim et al. (2016) also observed that retentate <3 kDa had the higher amount of free amino acids (630 mg/g protein). On the other hand, permeate <3 kDa presented a lower content than retentate <3 kDa which the authors associated with the high protein concentration in retentate which allowed the release of more amino acids during hydrolysis. In fact, the amino acids profile depends on the method and conditions of protein extraction applied to yeast (Jacob et al., 2019; B Podpora & Swiderski, 2015; Bartlomiej Podpora et al., 2016) and several authors have suggested their application in food market since the higher EAA content than FAO/WHO recommendations (Caballero-Córdoba & Sgarbieri, 2000; Jacob et al., 2019; B Podpora & Swiderski, 2015; Bartlomiej Podpora et al., 2016; Vieira et al., 2016) and the amount of promising amino acids to be applied in flavour enhancing (Amorim et al., 2016; Bartlomiej Podpora et al., 2016).

On the other hand, the discover of aspartic and glutamic acid, cysteine, histidine and lysine in Gpep and Mpep extracts, especially in free form (*Supplementary material 2*), shows potential for iron-binding to produce iron-peptide chelates as demonstrated by de la Hoz et al. (2014). These amino acids have functional groups capable of establishing coordinated covalent bonds and their considerable free amounts in fractions <1 kDa can make them iron-delivery components to produce food supplements targeted for anti-anaemic market.

3.2. Molecular weight profile

Generally, ultrafiltration membranes (1–50 kDa) are used to recover bioactive peptides and amino acids. On the other hand, nanofiltration



% MW		> 2000 Da	2000-1500 Da	1500-1000 Da	1000-500 Da	< 500 Da
Gpep	>1 kDa	7.81	9.50	21.7	45.7	15.3
	<1 kDa	5.83	2.14	4.02	36.9	51.1
Mpep	>1 kDa	19.5	19.1	24.5	26.7	10.2
	<1 kDa	5.47	4.31	5.57	36.8	47.9

Fig. 2. Peptides molecular weight distribution (% MW) in peptide-rich extracts under and above 1 kDa.

membranes (100–1000 Da) are widely used for low MW peptides purification processes by industry (Vollet Marson et al., 2020). In fact, peptides with biological activity are made up of 3–20 amino acids and their amino acid composition and sequence is responsible for their bioactivity (Amorim, Marques, et al., 2019).

As mentioned above, an ultrafiltration with 1 kDa cut-off membrane was performed to increase the protein concentration of initial waste stream supernatants. As expected, the fractions of Gpep and Mpep <1 kDa had approximately 88 and 85% of peptides under 1000 Da, respectively, with about 50% of these being under 500 Da. On other hand, the Gpep and Mpep fractions >1 kDa presented 39% and 63% of peptides above 1 kDa (Fig. 2). The unexpected large percentage of peptides under 1 kDa observed in Gpep >1 kDa fraction (61%) may be related with the limited selectively of ultrafiltration cut-off membranes due to fouling phenomena and critical flux, since they are directly related with transference phenomenon and protein interactions, which have been described as the main challenges of membrane filtration processes (Vollet Marson et al., 2020). Huang, Gao, Ma, and Lu (2012) maximized the protein yield from spent yeast using a 5 kDa PES ultrafiltration, applying a Box-Behnken design. The authors found that the optimal conditions operation (2.7% of yeast concentration, pH 5.0 and 14.0 psi of pressure) allowed a 95.0% of protein yield which underlines the importance of investigating these parameters to ensure a high-performance ultrafiltration process. In line with the present results, performing a 5 kDa ultrafiltration after enzymatic hydrolysis, Hu et al. (2014) obtained a yeast peptide hydrolysate with 80.41% peptides <1 kDa, followed by 18.26% of 1–3 kDa and some residues between 3 and 5 kDa. By applying a sequential filtration membrane process using 50–8-1 and 15–8-1 kDa to spent yeast hydrolysates, Marson et al. (2022) observed a gradual change of peptides MW: 50% of peptides <1 kDa on the first retentates (50 and 15 kDa), followed by 65% on the second (8 kDa) and 80% on the last (1 kDa), finishing with 90% < 1 kDa on the final permeate. The first retentates had a maximum of 19% of peptides with higher MW (> 7 kDa).

3.3. Biological activity

3.3.1. In vitro cytotoxicity assessment

Considering the application of the present peptide-rich extracts in nutraceutical market, Caco-2 cell line was used to evaluate their cytotoxic effect, with the objective of finding a non-cytotoxic concentration for performing the following bioactivities assays. In fact, Caco-2 cell line is the most widely used *in vitro* model for studies of permeability, transport and absorption of substances since they are derived from human colorectal adenocarcinoma and present high morphological and physiological similarity to the human enterocyte, thus being representative of the human intestinal barrier, and, to a lesser extent, to the human intestine (Osakwe, 2016).

Caco-2 were treated over a period of 24 h with increased concentrations of the peptide-rich extracts obtained from 0.8 to 5 mg/mL. As described in ISO 10993-5:2009 (ISO, 2009), an ingredient may have a cytotoxic potential if cell viability is reduced >30% of the non-treated cells. PrestoBlue ® was used as a cell viability indicator since when added to living cells it is metabolically reduced, becoming highly fluorescent. The number of viable cells, or metabolically active, correlates to the fluorescence intensity measured (Invitrogen, 2010).

The Caco-2 metabolism was inhibited >30% as of 2.5 mg/mL of Gpep extracts which means that equal and above concentrations cannot be used in following biological assays due to potential cytotoxic (*Supplementary material 3*). On the other hand, Mpep <1 kDa and > 1 kDa

Table 3
IC50 values (mg/mL) at ACE-inhibition assay and ABTS and DPPH scavenging activity of peptide-rich extracts (expressed as μmol Trolox Equivalent/mg of protein and μmol Trolox Equivalent/g of extract).

		*ACE (mg/mL)**	*ABTS (μmol TE)**		*DPPH (μmol TE)**	
		IC50	*mg protein*	*g extract*	*mg protein*	*g extract*
Gpep	> 1 kDa	1.54 ± 0.04	520 ± 181	337 ± 123	161 ± 39	110 ± 26
	< 1 kDa	0.99 ± 0.04	754 ± 186	450 ± 115	170 ± 47	114 ± 31
Mpep	> 1 kDa	1.43 ± 0.07	563 ± 176	416 ± 166	145 ± 45	125 ± 39
	< 1 kDa	1.72 ± 0.11	1098 ± 238	492 ± 109	270 ± 66	133 ± 32

Results are expressed in average ± standard deviation (n = 2). TE - Trolox Equivalent. *For Caco-2 biocompatible extract concentration. For each assay, no statistically significant differences ($p < 0.05$) were found between peptide rich-fractions.

had a completely different behaviour from each other since all the tested concentrations of Mpep <1 kDa did not inhibit cell metabolism, whereas Mpep >1 kDa decreased Caco-2 metabolism at concentrations higher than 5 mg/mL. The differences observed may be associated to peptides structure and characteristics such as charge, amino acids profile and sequence; besides, other unknown components may also be present in peptide-rich extracts. Regarding these results, the peptide-rich extracts concentrations chosen for the bioactivities were: Gpep >1 kDa and < 1 kDa - 1.25 mg/mL, and Mpep >1 kDa – 2.50 mg/mL and Mpep <1 kDa – 5.00 mg/mL since they appeared not to change cells viability >30% as mentioned in ISO 10993-5:2009 (ISO, 2009).

Mirzaei et al. (2019) found the fractions 3–5 kDa (0.5 and 1 mg/mL) and < 3 kDa (0.5–2 mg/mL) did not decrease significantly the Caco-2 cell viability at 24 h exposure. In fact, they reported non-cytotoxic concentrations below 2 mg/mL which is in according to the current study, which observed Mpep <1 kDa being non-cytotoxic below 5 mg/mL. However, these differences are strongly dependent on the methods used for production of yeast protein.

3.3.2. Antihypertensive activity

The potential antihypertensive proprieties of produced peptide-rich extracts were evaluated using the *in vitro* ACE-inhibitory assay and the results were presented in Table 3. ACE is a zinc-dependent dipeptidyl carboxypeptidase that catalyses the conversion of angiotensin I to the potent vasoconstrictor angiotensin II which is responsible for contraction of muscles surrounding blood vessels, increasing their inner pressure, and causing high blood pressure. For this reason, the inhibition of ACE activity leads to the decrease of angiotensin II, resulting in expanding blood vessels and consequently to blood pressure decrease, preventing the development of cardiovascular diseases (Z. Liu, 2007). The peptide rich-extracts have IC50 values from 0.99 to 1.72 mg/mL for ACE-inhibition, with Gpep <1 kDa having the greatest antihypertensive effect (IC50: 0.99 ± 0.04 mg/mL). The low MW of peptides of Gpep <1 (36.9% 1000–500 Da, 51.1% < 500 Da), in comparison with fractions >1 kDa, can be related with the antihypertensive activity as described by Amorim et al. (2016). Hu et al. (2014) observed a strong ACE inhibitory activity (IC50: 29.32 μg/mL) in permeate of 5 kDa ultrafiltration yeast hydrolysate, containing about 80% of peptides <1 kDa. Amorim, Pinheiro, and Pintado (2019) also showed a high ACE-inhibitory activity in permeates <3 kDa from yeast hydrolysates (84.2 μg/mL) when compared with the respective retentate fractions (258 μg/mL). However, the same result was not verified in Mpep <1 kDa (IC50: 1.72 ± 0.11 mg/mL) that had a similar MW profile (36.8% 1000–500 Da, 47.9% < 500 Da) with Gpep <1 kDa (Fig. 2). In this case, the lower ACE-inhibition can be influenced by the reduced amount of protein (48.3 ± 15.9%) and amino acids (254 ± 85 mg/g protein) in comparison with the other three fractions (Table 1, Supplementary material 2). The high amount of hydrophobic and basic amino acids of yeast protein are some of the pointed reasons for the ACE-inhibition activity observed. Some authors proposed that hydrophobic amino acids located at C-terminal of small peptides (2 to 20 amino acid residues) might influence their binding to the ACE active site, suggesting a potential structure-activity relationship. Mirzaei et al. (2015) suggested that the ACE inhibition activity found in yeast trypsin protein hydrolysates (IC50: 0.84 mg/mL) was due to the total content of hydrophobic amino acids and the peptides <3 kDa were responsible for the strongest ACE inhibitory effect (IC50 = 0.32 mg/mL) of the fraction <3 kDa due to the low MW, which increases accessibility and better binding to the ACE active site. Huang, Wang, Hou, and Hu (2020) attributed the strong *in vitro* ACE-inhibitory activity (IC50: 26.13 μg/mL) of a yeast protein hydrolysate to the large number of peptides under 1.5 kDa (91.2%). One important parameter for efficacy might be related with peptide's bioavailability in human body, since digestion and absorption processes change their integrity, influencing their binding with ACE and compromising their bioactivity (Xue, Yin, Howell, & Zhang, 2021). The range of IC50 obtained in the present study is in agreement with previously reported studies about ACE inhibitory potential of yeast protein extracts (Mirzaei et al., 2015). Depending on the protein extraction method applied to yeast, Mirzaei et al. (2015) obtained a range of 0.32 to 2.70 mg/L of IC50 for peptide fractions after 10, 5 and 3 kDa ultrafiltration. Comparing their ACE-inhibition results of fractions <3 kDa, that have the most similar MW of Gpep and Mpep extracts, the present study obtained intermediate IC50 values (0.99–1.72 mg/mL) (Table 3) between hydrolysates and autolysates since they show about 0.32–0.79 mg/L and 2.7 mg/L, respectively.

3.3.3. Antioxidant activity: $ABTS^+$ and DPPH

The potential antioxidant activity of peptide-rich extracts produced in the present study was demonstrated by two scavenging-based assays and the results were expressed in Table 3. Even though $ABTS^+$ and DPPH methods involved redox mechanisms, their reagents, products and transfer reactions are slightly different. The $ABTS^+$ scavenging activity is based on the reduction of cation-radical $ABTS^+$ into colourless the ABTS by electron transference of an antioxidant. On the other hand, DPPH can be reduced by hydrogen atom donation of an antioxidant, changing the colour of final product from pinkish to pale yellow. Both changes can be measured spectrophotometrically (Dasgupta & Klein, 2014; Stratil, Klejdus, & Kubáň, 2006).

All peptide-rich extracts demonstrated the ability to reduce the cationic radical $ABTS^+$ with concentrations of TE from 337 to 492 μmol/g extract, with a tendency of higher scavenging activity in fractions <1 kDa (Gpep: 450 μmol TE/g, Mpep: 492 μmol TE/g in relation to >1 kDa (Gpep: 337 μmol TE/g, Mpep: 416 μmol TE/g). The same tendency was observed with DPPH results, where all extracts demonstrated the ability to reduce DPPH in a range of 110 to 133 μmol TE/g (Table 3). The relationship between peptides MW and free radical scavenging activity was already investigated since peptide fractions from selenium-rich yeast hydrolysate with MW < 1 kDa showed higher ABTS $^+$, DPPH, OH and O_2^- scavenging activity in relation to fractions <3 kDa (Guo et al., 2020).

Comparing $ABTS^+$ and DPPH methods, DPPH provided lower values of TE than $ABTS^+$ which it was already described in literature since DPPH radical is much more stable than $ABTS^+$ (Mareček et al., 2017). A similar result was found by Guo et al. (2020) since they obtained a strong $ABTS^+$ scavenging ability from all yeast protein hydrolysates and the same was not observed for scavenge DPPH. Although the high reactivity of ABTS can be advantageous, since it can react with a wider range of antioxidants, the instability of $ABTS^+$ solution can lead to unbiased results, and thus is important the support results with a second scavenging activity assay, like DPPH. Regarding this issue, Mirzaei et al. (2015) demonstrated the strong scavenging activities by DPPH and $ABTS^+$ assays of yeast protein hydrolysates and peptide fractions. DPPH values of peptide fractions <3 kDa, which are closer of Gpep and Mpep MW peptides, ranging from 50 to 489 μM TE/mg protein, which are in in agreement with the present study results (145–270 μM TE/mg protein). In fact, the authors pointed that the large range of DPPH findings was dependent on protein extraction method applied to the yeast. For $ABTS^+$ scavenging assay, Mirzaei et al. (2015) showed values about 2500 to 8000 μM TE/mg protein which were slightly higher than the present results (520–1098 μM TE/mg protein). Likewise Podpora et al. (2016) obtained higher $ABTS^+$ scavenging activity (4615 to 5069 μM TE/g extract) for protein hydrolysates, containing large amounts of free amino acids and peptides from 703 to 1740 Da, than the current investigation. However, the authors related these results with the presence of phenolic compounds, which were not evaluated in the present study. Furthermore, and as mentioned above, these differences can be related with the $ABTS^+$ solution instability and the method used for yeast protein extraction. On the other hand, the DPPH scavenging activity obtained in the current investigation was stronger than those found by Marson et al. (2020) in spent yeast hydrolysates with MW about 35 kDa, which can be related with the lower MW of peptides in Gpep and Mpep.

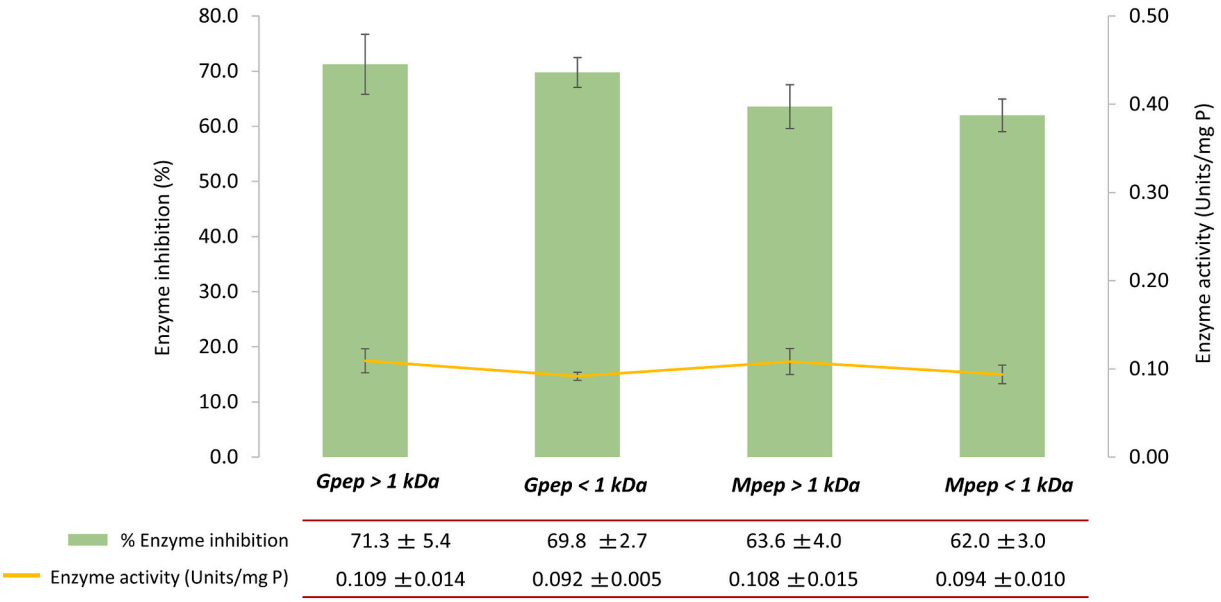


Fig. 3. HMG-CoA reductase inhibition (%) and activity (Units/mg protein) for the peptide-rich extracts. Results are expressed in average ± standard deviation. No statistically significant differences ($p < 0.05$) were found between peptide rich-fractions.

Proving the strong antioxidant potential of spent yeast peptides, other studies have demonstrated their influence in *in vitro* repair pathways against oxidative stress caused by H_2O_2 or UV radiation (Butylina, Shataeva, & Nyström, 2007; Mirzaei et al., 2019), which involve mechanisms of lipid peroxidation and protein oxidation inhibition (Mirzaei et al., 2019). For this reason, yeast peptides were recently described as promising cosmeceutical additives, revealing *in vitro* and *in vivo* antioxidative and skin oxidative damage protection properties (Guo et al., 2020).

Regarding the importance of combined effects in one single product as functional ingredient for food industry, several authors studied the antioxidant properties of spent yeast peptides together with ACE and/or α-glucosidase inhibition and immunostimulant activities (Amorim, Marques, et al., 2019; Hassan, 2011; Jung et al., 2011; Mirzaei et al., 2015; Vieira, Melo, & Ferreira, 2017). Actually, the antioxidant activity has been linked to the prevalence of chronic disorders, such as hypertension, diabetes, aging, cancer, and neurodegenerative disorders (Amorim, Marques, et al., 2019). In the present study, regarding the ACE-inhibition and scavenging activity results, in general, all Gpep and Mpep fractions showed to be good bioactive ingredients to be potentially incorporated in functional food and commercialized at nutraceutical market (Table 3).

From all peptide fractions, Gpep <1 kDa slightly stood out with the lowest IC50 value of ACE-inhibition (0.99 mg/mL) together with a strong (second highest) antioxidant capacity in ABTS (450 μM TE/g extract) and DPPH (144 μM TE/g).

3.3.4. *Anti-cholesterolemic activity*

Together with antihypertensive and antioxidant proprieties, the potential effect of Gpep and Mpep fractions on cholesterol-lowering was assessed by HMG-CoA reductase inhibition assay (Fig. 3). The HMG-CoA reductase has been considered a major target for the treatment of hypercholesterolemia since it is a key enzyme in cholesterol biosynthesis by mevalonate pathway where it is produced from acetyl-CoA. Bioactive peptides have influenced the lipid profile of blood (Hajfathalian, Ghelichi, García-Moreno, Moltke Sørensen, & Jacobsen, 2018), being described as a novel class of HMG-CoA reductase inhibitors that can directly interact with this enzyme to block the mevalonate pathway and prevent hypercholesterolemia (Lin, Chang, Chou, Huang, & Shiuan, 2015).

To the authors knowledge, the present study was the first to demonstrate the effect of spent yeast peptides on HMG-CoA reductase inhibition. All peptide fractions inhibited HMG-CoA activity from 62.0 to 71.3% (Fig. 3). No significant differences were found between fractions under and above 1 kDa and between the type of waste stream used (Gpep *vs.* Mpep).

Comparing the current results with literature, the percentages of HMG-CoA reductase inhibition of Gpep and Mpep were higher than *Amaranthus cruentus* protein hydrolysate <3 kDa (45%) which comprised the amino acid sequences GGV, IVG/LVG and VGVI/VGVL (Aparecida, Soares, Mendonça, Ívini, & Castro, 2015). In another study, peptides <3 kDa from faba and adzuki bean revealed about 60 to 80% of HMG-CoA reductase inhibition which are in accordance with our results (Ashraf et al., 2020).

Regarding the obesity issue that can be associated with high cholesterol levels and lipid metabolic disorders, other studies have previously explored the anti-obesity effect of yeast hydrolysates but the effect on cholesterol pathway was not studied (Jung et al., 2014; Kim et al., 2012; Park et al., 2013). Further studies on the subject are need to draw further conclusions.

4. Conclusion

The present study demonstrated the ability to produce peptide-rich extracts from waste streams of spent yeast β-glucans and mannans extraction processes by 1 kDa ultrafiltration technology. The results showed that yeast extracts with high protein content and EAA, comprising about 1 kDa MW peptides, were capable of strong ACE and HMG-CoA reductase inhibition, and $ABTS^{+}$ and DPPH reduction, indicating their antihypertensive, anti-cholesterolemic and antioxidant capacities. In this way, the peptide-rich extracts produced showed to be attractive to be incorporated into capsules, tablets or other kind of formulations towards commercialization at the nutraceutical market for human supplementation. On the other hand, their high amount of glutamic acid also makes them promising ingredients to be applied as flavour enhancers.

CRediT authorship contribution statement

Ana Sofia Oliveira: Conceptualization, Investigation, Writing –

original draft. **Joana Odila Pereira:** Investigation, Writing – review & editing. **Carlos Ferreira:** Investigation, Writing – review & editing. **Margarida Faustino:** Investigation. **Joana Durão:** Investigation. **Manuela E. Pintado:** Resources, Supervision, Project administration, Funding acquisition. **Ana P. Carvalho:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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